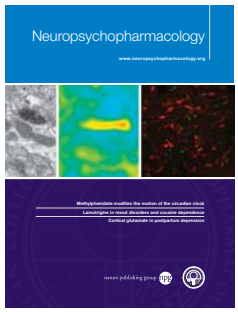

Presynaptic Dopamine Capacity in Patients with Treatment Resistant Schizophrenia Taking Clozapine: An [^{18}F]DOPA PET Study

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Cite this article as: Euitae Kim, Oliver D Howes, Mattia Veronese, Katherine Beck, Seongho Seo, Jin Woo Park, Jae Sung Lee, Yun-Sang Lee, Jun Soo Kwon, Presynaptic Dopamine Capacity in Patients with Treatment Resistant Schizophrenia Taking Clozapine: An [^{18}F]DOPA PET Study, *Neuropsychopharmacology* accepted article preview 18 November 2016; doi: 10.1038/npp.2016.258.

Received 18 June 2016; revised 9 November 2016; accepted 11 November 2016; Accepted article preview online 18 November 2016

Presynaptic dopamine capacity in patients with treatment resistant schizophrenia taking clozapine: an [^{18}F]DOPA PET study

Treatment resistant schizophrenia

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Word counts

Abstract: 248

Text body: 3862

Abstract

Some patients with schizophrenia show poor response to first-line antipsychotic treatments and this is termed treatment resistant schizophrenia. The differential response to first-line antipsychotic drugs may reflect a different underlying neurobiology. Indeed, a previous study found dopamine synthesis capacity was significantly lower in patients with treatment resistant schizophrenia. However, in this study, the treatment resistant patients were highly symptomatic whilst the responsive patients showed no or minimal symptoms. The study could not distinguish whether this was a trait effect or reflected the difference in symptom levels. Thus we aimed to test whether dopaminergic function is altered in patients with a history of treatment resistance to first-line drugs relative to treatment responders when both groups are matched for symptom severity levels by recruiting treatment resistant patients currently showed low symptom severity with the clozapine treatment.

Healthy controls(n=12), patients treated with clozapine(n=12) who had not responded to first-line antipsychotics and patients who had responded to first-line antipsychotics(n=12) were recruited. Participants were matched for age and sex, and symptomatic severity level in patient groups. Participants' dopamine synthesis capacity was measured by using [^{18}F]DOPA PET.

We found that patients treated with clozapine show lower dopamine synthesis capacity than patients who have responded to first-line treatment(Cohen's d=0.9191(whole striatum), 0.7781(Associative striatum), 1.0344(Limbic striatum), 1.0189(Sensorimotor striatum) in line with the hypothesis that the dopaminergic function is linked to treatment response.

This suggests that a different neurobiology may underlie treatment resistant schizophrenia

and that dopamine synthesis capacity may be a useful biomarker to predict treatment responsiveness.

Introduction

A recent meta-analysis of presynaptic dopaminergic function as well as studies of dopamine receptor and transporter levels, identified elevated presynaptic dopamine synthesis and release capacity as the primary locus of dopaminergic abnormality in schizophrenia, with a large effect size (Howes *et al*, 2012a). Further support for this comes from studies of people at risk of psychosis that have also found elevated dopamine synthesis and release capacity (Howes *et al*, 2009b; Mizrahi *et al*, 2012; Stone *et al*, 2010), and studies linking this to the later onset of psychosis (Howes *et al*, 2011a; Howes *et al*, 2011b).

Antipsychotic drugs are central to the treatment of schizophrenia (Howes and Murray, 2014). All current antipsychotic drugs block dopamine receptors (Howes *et al*, 2009a) and their affinity for dopamine receptors is directly associated with their clinical effectiveness (Creese *et al*, 1976; Howes *et al*, 2009a). Furthermore, dopamine receptor blockade has been shown to be necessary for clinical response (Kapur *et al*, 2000; Nordstrom *et al*, 1993), and greater presynaptic dopamine dysfunction at baseline is associated with greater subsequent response to antipsychotic treatment (Abi-Dargham *et al*, 2000). Coupled with the evidence of elevated dopamine synthesis and release capacity in schizophrenia, this indicates that antipsychotics work by blocking the consequences of elevated dopaminergic neurotransmission (Howes *et al*, 2009a).

However, 15%-30% of patients with schizophrenia do not experience a significant reduction in symptoms with standard, first-line antipsychotic treatment (Kane *et al*, 1988). These patients are considered to be treatment resistant, defined as an inadequate response to adequate treatment trials with at least two different first-line antipsychotic drugs (Beck *et al*, 2014; Kane, 1989). Patients with treatment resistant schizophrenia are highly unlikely to

respond to further treatment with first-line antipsychotic drugs (Suzuki *et al*, 2007). Clozapine is the only antipsychotic drug with proven efficacy in patients with treatment resistant schizophrenia (Agid *et al*, 2013; Kumra *et al*, 2008). However, clozapine's use is limited by poor tolerability in some patients and a complex monitoring regime (Howes *et al*, 2012b). There is, therefore, a need to understand the neurobiology of treatment resistant schizophrenia better to develop alternative treatments to clozapine.

It has been proposed that the differential response to first-line antipsychotic drugs seen in patients reflects a different underlying neurobiology, and, specifically, that patients with treatment responsive schizophrenia show elevated dopamine synthesis and release capacity, which is not seen in patients with treatment resistant schizophrenia (Howes and Kapur, 2014). Supporting this, studies of plasma dopamine metabolites show that patients with lower baseline levels are less likely to respond to first-line antipsychotic drugs (Ottong and Garver, 1997; Yoshimura *et al*, 2003). Furthermore, a post-mortem study comparing dopaminergic markers between patients who had histories of good and poor response found that patients with a history of poor response showed fewer dopaminergic synapses identified by the immunochemical localization of tyrosine hydroxylase (Roberts *et al*, 2009). There is also evidence from a PET study that found dopamine synthesis capacity was significantly lower in patients with treatment resistant schizophrenia, who remained highly symptomatic and functionally impaired despite adequate treatment trials with at least two different first-line antipsychotic drugs, when compared to patients who had responded to first-line antipsychotic treatment (Demjaha *et al*, 2012). Taken together with the previous literature that there is a positive association between symptomatic severity and dopamine synthesis capacity in schizophrenia (Howes *et al*, 2007), this finding suggests that dopaminergic function is

different in treatment resistant schizophrenia. However, in this study, the treatment resistant patients were highly symptomatic whilst the responsive patients showed no or minimal symptoms. The study could not, therefore, distinguish whether this was a trait effect or a reflection of the difference in symptom levels.

Thus, we aimed to test whether dopaminergic function is altered in patients with a history of treatment resistance to first-line drugs relative to treatment responders when both groups are matched for symptom severity levels. In line with the proposal that treatment resistant schizophrenia has a non-dopaminergic basis (Howes and Kapur, 2014), we hypothesized that patients with treatment resistant schizophrenia would have lower dopamine synthesis capacity compared to patients who have responded to first-line antipsychotic drugs when the symptom state is matched. To match the symptom state, we recruited patients with treatment resistant schizophrenia who had responded to clozapine and compared them to patients who had responded to first-line antipsychotic drugs using [^{18}F]DOPA PET to measure presynaptic dopamine synthesis capacity. Additionally, we included a matched healthy volunteer group to provide a comparison with normal dopamine synthesis capacity.

Materials and Methods

This study was approved by the Institutional Review Board of Seoul National University Hospital, Seoul, Korea and was carried out in accordance with the Helsinki Declaration of 1975, as revised in 2008.

Participants

Participants (aged 18 to 65 years) received a full explanation of the study and provided written informed consent to participate. Screening procedures included physical examination,

checking vital signs, laboratory tests (hematology, blood chemistry, and urinalysis), and a 12-lead electrocardiogram. Subjects with any medically significant abnormalities and/or psychiatric diseases (except schizophrenia in patients group) were excluded. Symptoms were rated using the Positive and Negative Syndrome Scale (PANSS), and chlorpromazine equivalent doses for antipsychotic treatment were calculated using the formula described by Andreasen *et al* (2010).

Patients with schizophrenia

Patients were recruited from the outpatient clinic in the Seoul National University Hospital. Patients who met the following inclusion criteria were invited to participate in the study: (1) Patients who met DSM-IV criteria for schizophrenia (2) Patients who had a total score of less than or equal to 80 in the PANSS and no items with a score above 4 on the positive subscale of the PANSS (3) patients who have received first-line antipsychotic drugs including risperidone, olanzapine and paliperidone (first-line AP group) or clozapine (clozapine group) for at least 12 weeks (4) The first-line AP group had to have no history of being given clozapine or being refractory to first-line antipsychotic drug treatments (5) Based on chart review, the clozapine group had to have history of no response to at least two different first-line antipsychotic drugs. Twelve patients were enrolled for each group.

Healthy controls

Twelve healthy controls (control group) were recruited via advertisement and were matched to the patients with schizophrenia on the basis of age (within 3 years) and sex. A psychiatric interview for the presence of DSM-IV axis I disorders was conducted using the Structured Clinical Interview for DSM-IV Axis I Disorders, Non-Patient edition (SCID-I/NP) (First *et al*, 2002).

Positron Emission Tomography

All the patients except one who took a long-acting injectable risperidone were taking their antipsychotic drugs at night once a day. The patients were instructed to take their antipsychotic medication at 9 p.m. a day before the scan. The date of the PET scan was randomly assigned to participants to avoid a possible bias by consecutive scans from the same group. The PET scan was performed at 2 p.m. and participants were required to fast and abstain from smoking and drinking from midnight on the day of the scan and received 150 mg carbidopa and 400 mg entacapone orally 1 hour prior to scanning to reduce the formation of radiolabeled metabolites (Turkheimer *et al*, 1999).

Participants underwent a short computed tomography (CT) for attenuation correction and PET imaging on a Biograph 40 Truepoint PET/CT scanner (Siemens, Knoxville, Tennessee, USA) for 95 minutes after an intravenous bolus injection of approximately 370 MBq (10mCi) of [^{18}F]DOPA with minimum specific activity of 1.30 x 10^7 Ci/mol. Head movement was monitored with a mark and minimized using a light head strap. After routine corrections for uniformity, decay corrections and attenuation (using the CT), the PET imaging data acquired in a list mode were reconstructed with a filtered back-projection using a Gaussian filter. Images were collected in a three-dimensional mode with 148 axial slices, an image size of 256 x 256, a pixel size of 1.3364 x 1.3364 mm^2 and a slice thickness of 3 mm. The dynamic volumetric images were sequenced using the following framing: 2 × 30, 4 × 60, 3 × 120, 3 × 180, and 15 × 300 sec.

For the analysis of volume effects in the striatum, high-resolution T1-weighted magnetic resonance images (MRIs) were also acquired after the PET scan (TE = 1.89 ms, TR = 1670 ms, flip angle = 9°, 208 slices, matrix = 256 × 256, FOV = 250 mm).

Image analysis

PET image analysis was conducted as previously described (Bloomfield *et al*, 2014). Inter-frame correction for head movement during the scan was performed by denoising the non-attenuation-corrected dynamic images using a level 2, order 64 Battle-Lemarie wavelet filter. Frames were hence realigned to a single 'reference' frame, acquired 8 min post-injection, employing a mutual information algorithm (Turkheimer *et al*, 1999). The transformation parameters were then applied to the corresponding attenuated-corrected dynamic images, creating a movement-corrected dynamic image. Subsequently, the realigned images were spatially normalized by registering their summed image to the [^{18}F]DOPA template created in a previous study (McGowan *et al*, 2004). Region-of-interest (ROI) time-activity curves (TACs) were hence extracted using atlas maps for the whole striatum, and its associative, limbic, and sensorimotor sub-regions (Martinez *et al*, 2003). The cerebellum was used as the reference region as it is a region with minimal dopaminergic projections (Hammers *et al*, 2003). Finally, using the cerebellar TAC as a reference region input, the Gjedde-Patlak plot (Patlak and Blasberg, 1985) was applied at ROI and voxel level to derive the influx rate constants ($k_{i,}^{cer}$) relative to the cerebellum, for the regions of interest and individual parametric maps respectively. The analysis was performed using a combination of SPM5 package (http://www.fil.ion.ucl.ac.uk/spm) and in-house code based on Matlab2012b®(The Mathworks Inc., MA, USA). A previous test retest study has found this approach to have high reliability for striatum (Egerton *et al*, 2010). Average parametric images in each group were derived from the individual parametric images normalized into Montreal Neurological Institute standard space (matrix dimension: 91x109x91; voxel size: 2mm isotropic) using the participant's PET summation image and the [^{18}F]DOPA template.

Volumetric segmentation in each high-resolution T1 weighted MRI was performed using the FreeSurfer image analysis suite (http://surfer.nmr.mgh.harvard.edu/) (Fischl, 2012) to extract striatal volumes.

Statistical analysis

After confirming the data were normally distributed by using the Kolmogorov-Smirnov test, we used independent t-test and analysis of variance test to compare demographic variables and striatal volumes between groups. Pearson's Chi-square test was used to test difference in sex ratio between groups. A mixed effects model was employed in a repeated measures analysis to test whether there was a group effect on k_i^{cer} with the ROI (Region; modeled as a dummy variable: 1=associative, 2=limbic, 3=sensorimotor subregions) and the group (Group: modeled as a dummy variable: 1=control group, 2=first-line AP group, 3=clozapine group) as fixed effects and subjects as random effects. Pearson correlation analysis was employed to investigate the relationship between k_i^{cer} and symptoms measured by using PANSS and between the striatal volume and the duration of illness or duration of exposure to current antipsychotics.

Results

Twelve participants were recruited in each group. One smoking patient was enrolled in the clozapine group and there was no participant with a history of alcohol abuse and/or dependence. Table 1 shows demographic characteristics of participants. The mean age (±s.d.) of participants was 30.9±8.6 years. There was no significant difference in age between the groups (F=0.05, df=2,33, p=0.951). The gender distribution was not significantly different

between groups (χ^2=0.262, df=2, p=0.877). The mean PANSS total score (±s.d.) was 31.7±1.1 in control group, 50.3±11.1 in the first-line AP group and 49.7±7.9 in the clozapine group. As expected, there was a significant effect of group on PANSS scores (F=21.75, df=2, 33, p<0.001). Pairwise comparison using Bonferroni's correction revealed significantly lower scores in the control group than in the first-line AP group (mean difference=18.67, s.e.=3.21, df=33, p<0.001) and in the clozapine group (mean difference=18.00, s.e.=3.21, df=33, p<0.001), but no significant difference in the total PANSS score between the first-line AP group and the clozapine group (mean difference=0.67, s.e.=3.21, df=33, p=1.000). Antipsychotic drugs in the first-line AP group were risperidone for 5 patients, paliperidone for 3 patients and olanzapine for 4 patients. The mean chlorpromazine-equivalent doses (±s.d.) were 285.4±153.2 mg in the first-line AP group and 261.4±117.5 mg in the clozapine group. The chlorpromazine equivalent doses were not significantly different between the first-line AP group and the clozapine group (t=-0.43, df=22, p=0.671).

The mean striatal volumes (±s.d.) were 21953.6±1896.3 mm^3 in the control group, 23685.6±2787.8 mm^3 in the first-line AP group and 22576.8±2529.8 mm^3 in the clozapine group, and there was no significant difference in the striatal volume between the groups (F=1.56, df=2, 33, p=0.225). There was no correlation between the striatal volume and the duration of illness (r=0.066, p=0.756) or duration of exposure to current antipsychotics (r=-0.104, p=0.627). The mean injected dose (±s.d.) of [^{18}F]DOPA was 10.0±0.7 mCi in the control group, 9.8±0.9 mCi in the first-line AP group and 9.9±0.7 mCi in the clozapine group and there was no significant difference in the injected dose between the groups (F=0.33, df=2, 33, p=0.719). Figure 1A shows average parametric images of [^{18}F]DOPA (k_i^{cer}) in each group and table 2 shows k_i^{cer} values. The k_i^{cer} values were significantly different according to the

group (Group: F=15.96, df=2,95.0, p<0.001; Region: F=19.46, df=2,63.7, p<0.001) (Figure 2). The k_i^{cer} difference according to the group was still significant after incorporating the striatal volume as a covariate (Group: F=12.88, df=2, 94.4, p<0.001; Region: F=34.69, df=2, 59.95, p<0.001). In pairwise comparison, the clozapine group showed significantly lower k_i^{cer} value than the control group (mean difference=-0.00154, s.e.=0.00028, df=95.0, p<0.001) and the first-line AP group (mean difference=-0.00110, s.e.=0.00028, df=95.0, p<0.001) (Figure 1B). However, the k_i^{cer} value in the first-line AP group was not significantly different from that in the control group (mean difference=-0.00044, s.e.=0.00028, df=95.0, p=0.367) (Figure 2). There was no correlation between k_i^{cer} from the whole striatum and the total scores of PANSS in either patient group (r=0.244, p=0.445 for first line AP group; and r=-0.231, p=0.470 for clozapine group).

Discussion

Our study is the first to report dopamine synthesis capacity in a group of patients solely treated with clozapine. Elkashef *et al* (2000) also measured dopamine synthesis capacity in patients treated with clozapine. However, they reported the mean value of dopamine synthesis capacity in a group including patients treated with either first-line antipsychotic drugs or clozapine (Table 3). Our main finding is that patients who have responded to clozapine with the history of treatment resistance show lower dopamine synthesis capacity than patients who have responded to first-line treatment in line with the hypothesis that the dopaminergic function is linked to treatment response (Howes and Kapur, 2014). Both patient groups showed low symptom severity levels and were well matched for total symptom severity ratings. In view of this, our finding extends a previous study where symptom severity differed between treatment resistant and responsive groups (Demjaha *et al*, 2012), to indicate

that lower dopamine synthesis capacity is likely to reflect a trait, rather than state, aspect of treatment resistant schizophrenia. This is consistent with the hypothesis that treatment resistant schizophrenia has a different neurobiological basis to schizophrenia that has responded to first-line antipsychotic treatment (Howes and Kapur, 2014).

We observed no difference in dopamine synthesis capacity between the first-line AP group and the control group, in contrast to the majority of previous radiolabeled DOPA PET studies in schizophrenia, which report an elevation in schizophrenia (Table 3). It could be due to small sample size to detect difference in dopamine synthesis capacity between schizophrenia and controls. Our study was powered (power>0.8) to detect the difference between treatment resistant and responsive patients based on the effect size>1.2 reported in the only prior study (Demjaha *et al*, 2012). The Cohen's d effect size difference in dopamine synthesis capacity between schizophrenia and controls was 0.8 (Howes *et al*, 2012a). Thus the sample size might be small to detect differences between controls and schizophrenia. Another possibility is that it reflects a state effect in this group, either due to phase of illness or to antipsychotic treatment (Grace, 1992; Grace and Bunney, 1986; Grunder *et al*, 2003; Vernaleken *et al*, 2006). Most studies using radiolabeled DOPA were conducted in drug-naïve or drug-free patients and reported consistently higher levels of presynaptic dopamine function in schizophrenia compared with healthy controls (Table 3). In contrast, studies in patients treated with antipsychotic drugs showed mixed results. The primary outcome in the current study was the influx rate constant normalized to the distribution volume of the cerebellum. The blood flow changes induced by antipsychotic treatment have been reported in the frontal cortex (Miller *et al*, 2001), basal ganglia (Miller *et al*, 1997) and hippocampus (Medoff *et al*, 2001). However, the results are inconsistent in this aspect and the cerebellum, which served

as the reference region in our study, showed no blow flow change associated with antipsychotic treatment (Miller *et al*, 2001). Thus antipsychotic treatment is unlikely to affect the primary outcome by changing the distribution volume of the cerebellum. To our knowledge, there have been five studies which examined dopamine synthesis capacity in patients treated with antipsychotic drugs and compared it with healthy controls (Table 3) (Demjaha *et al*, 2012; Elkashef *et al*, 2000; Howes *et al*, 2013; McGowan *et al*, 2004; Shotbolt *et al*, 2011). Two reported higher level of presynaptic dopamine function in patients (Howes *et al*, 2013; McGowan *et al*, 2004), and two reported no difference between patients treated with antipsychotic drugs and healthy controls (Elkashef *et al*, 2000; Shotbolt *et al*, 2011) and Demjaha *et al* (2012) reported higher level of presynaptic dopamine function in antipsychotic responders and no difference in antipsychotic-refractory patients. The patients included in these studies were similar to our patient groups in being more chronic and less symptomatic than the studies of drug-naïve/free patients. There is some evidence that presynaptic dopamine dysfunction varies with phase of illness, increasing with acute psychosis (Howes *et al*, 2011a; Laruelle *et al*, 1999). Thus taken together, this inconsistency in the findings in chronic treated patients may be due to an effect of treatment and/or state of illness, which requires a prospective study to address this issue.

We found that the clozapine group showed a significantly lower k_i^{cer} value than the control group, with an effect size (Cohen's d) of 1.3. This is in contrast to the previous report by Demjaha *et al* (2012), which showed no difference in dopamine synthesis capacity between the healthy controls and the treatment-resistant patients with schizophrenia. A difference between the studies is that the treatment resistant patients in our study were treated with clozapine. This is the first study reporting lower dopamine capacity in patients treated with

clozapine. Clozapine is reported to reduce extracellular dopamine level after chronic administration (Shilliam and Dawson, 2005). This may account for the difference between the study by Demjaha *et al* (2012) and our study, though the mechanism of action reducing dopamine synthesis still remains obscure. It is also important to note that clozapine is reported to increase the activity of aromatic L-amino acid decarboxylase, the enzyme that convert *l*-DOPA (and radiolabeled DOPA in the PET scan) to dopamine (Neff *et al*, 2006). This could influence k_i^{cer} values in the clozapine group, although increased enzyme activity would be expected to increase k_i^{cer} values, whereas we saw lower k_i^{cer} in the clozapine group.

Clinical implications

Lower presynaptic dopamine capacity observed in the clozapine group may reflect a different underlying pathophysiology of schizophrenia in patients resistant to first-line antipsychotic drugs. Evidence that glutamate levels are higher in treatment resistant patients (Demjaha *et al*, 2014; Mouchlianitis *et al*, doi:10.1093/schbul/sbv151), suggests that glutamatergic differences could underlie the pathophysiology of treatment resistance, and is consistent with findings that clozapine acts on the glutamate system (Duncan *et al*, 1998; Javitt *et al*, 2005; Lopez-Gil *et al*, 2007). Other neurochemical systems may also be involved in treatment resistance (Selvaraj *et al*, 2014).

Furthermore, the finding supports the early initiation of clozapine treatment as it suggests there is less neurochemical rational for dopaminergic blockade. Reducing unnecessary exposure to first-line antipsychotic drugs could lower the risk of adverse effects as well. There is already some clinical evidence that clozapine initiation soon after the first episode of psychosis improves outcome (Agid *et al*, 2007; Remington *et al*, 2013). Our findings suggest that dopamine synthesis capacity may be a useful biomarker for predicting treatment

resistance, although sensitivity and specificity will need to be tested (Bose *et al*, 2008).

Limitations

This is a cross-sectional study and we did not measure presynaptic dopamine capacity before the administration of antipsychotic drugs or from illness onset. Though our result is consistent with lower dopamine synthesis capacity being a trait of treatment resistant schizophrenia, a prospective study is required for confirmation. Though it was not statistically significant, the average duration of exposure to current antipsychotic drugs was longer in the clozapine group than in the first-line AP group (Table 1). Moreover, the clozapine group had a period of treatment with first-line antipsychotic medication prior to clozapine treatment. As such, it is not unexpected that the patients in the clozapine group might have longer exposure to antipsychotics, though they did not respond to first-line antipsychotic drugs. Considering that the duration of exposure to antipsychotic drugs may affect dopamine synthesis capacity (Grace, 1992; Shilliam and Dawson, 2005), this needs to be taken into consideration when interpreting the result. According to the inclusion/exclusion criteria, we excluded participants with history of drug abuse or dependence. However, one smoking patient was enrolled in the clozapine group. There are some reports that cigarette smoking can influence dopamine synthesis capacity, though the results are inconsistent (Bloomfield *et al*, 2014; Rademacher *et al*, 2015; Salokangas *et al*, 2000). Nonetheless, re-analysis without the data from the patient with the history of cigarette smoking in the clozapine group found the same results and therefore smoking is unlikely to affect the result. Concomitant medication could have affected the outcome. We did not take the concomitant medication into consideration when interpreting results. This could be a limitation for the study. However, as seen in Table 1, the concomitant medication does not seem different

between two patient groups, hence is unlikely to affect the group difference in the outcome. The specific activity of [^{18}F]DOPA was not routinely measured in the current study and we were not able to compare the specific activity between groups. The difference in specific activity between groups could have affected the result. However, we randomly assigned the date of scan to participants to avoid the possible bias and visual inspection into individual data acquired on the same day found similar trends of lower k_i^{cer} values in the clozapine group than in the first-line AP group, which may suggest the bias is unlikely to affect the result.

Conclusions

Dopamine synthesis capacity is lower on average in patients resistant to first-line antipsychotic drugs relative to patients who have responded to first-line antipsychotic drugs, suggesting that a different neurobiology may underlie treatment resistant schizophrenia and that dopamine synthesis capacity may be a useful biomarker to predict treatment responsiveness.

Funding and Disclosure

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2016R1E1A1A02921618), SK Healthconnect (34-2013-0030) and grant no. NRF-2015R1C1A1A01054583 from the NRF. The authors have nothing to disclose.

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Figure Legends

Figure 1. Average parametric images (A) of [^{18}F]DOPA (k_i^{cer}) from the healthy controls (Control group), and patients treated with the first-line antipsychotic drugs (First-line AP group) or clozapine (Clozapine group) and statistical parametric mapping outputs (B) comparing control group and clozapine group (Left) and first-line AP group and clozapine group (Right). The statistical parametric mapping outputs are thresholded at $p<0.001$ uncorrected and the blue hairline indicates the voxels with highest t value.

Figure 2. [^{18}F]DOPA k_i^{cer} values in the whole striatum according to the group. Each dot represents an individual k_i^{cer} value and each vertical bar indicates the mean and the standard deviation for the corresponding group. Asterisks indicate significant differences between two groups ($p<0.001$).

Table 1. Demographic characteristics of participants

	Control group, n=12	First-line AP group, n=12	Clozapine group, n=12	p value
Age (yr±s.d.)	30.3 ± 8.4	31.1 ± 9.8	31.3 ± 8.1	0.951
Sex (male/female)	8 / 4	8 / 4	9 / 3	0.877
PANSS total score (±s.d.)	31.7 ± 1.1	50.3 ± 11.1	49.7 ± 7.9	<0.001
Positive scale score	7.0 ± 0.3	10.8 ± 2.7	11.2 ± 2.3	<0.001
Negative scale score	7.2 ± 0.5	13.2 ± 5.2	12.8 ± 2.8	<0.001
General psychopathology score	17.3 ± 1.0	26.3 ± 6.0	25.5 ± 3.9	<0.001
Duration of illness (mon±s.d.)	-	111.3 ± 108.2	144.7 ± 77.8	0.394
Antipsychotics (n)	-	Risperidone (5) Paliperidone (3) Olanzapine (4)	Clozapine (12)	
Antipsychotic dose (mg±s.d.)	-	Risperidone: 4.0±1.5 Paliperidone: 8.0±1.7 Olanzapine: 11.9±12.1	282.3 ± 126.9	
Chlorpromazine equivalent dose (mg±s.d.)	-	285.4 ± 153.2	261.4 ± 117.5	0.671
Duration of exposure to current antipsychotics (mon±s.d.)	-	64.1 ± 18.2	76.3 ± 12.4	0.584
Concomitant mediation (n)	-	None (6) SSRI (2) Benzodiazepine (3) Antiparkinsonian agent (4)	None (4) SSRI (4) Benzodiazepine (4) Antiparkinsonian agent (3)	

The chlorpromazine equivalent dose was calculated based on the formula from Andreasen *et al* (2010)

One patient treated with risperidone was given long-acting injectable risperidone. The dose of long-acting injectable risperidone was converted to oral equivalent.(Bai *et al*, 2007)

SSRI includes escitalopram and fluoxetine in the first-line AP group and escitalopram, sertraline and fluoxetine in the clozapine group.

Benzodiazepine includes lorazepam and clonazepam in the first-line AP group and lorazepam,

alprazolam and clonazepam in the clozapine group.

Antiparkinsonian agent indicates medication for treating extrapyramidal symptoms including propranolol, benztropine and trihexyphenidyl in both groups.

Table 2. [^{18}F]DOPA k_i^{cer} values (min^{-1}) (±s.d.) of regions-of-interest in the healthy controls (Control group) and patients treated with first-line antipsychotic drugs (First-line AP group) or clozapine (Clozapine group).

	Control group	**First-line AP group**	**Clozapine group**	**Cohen's *d* between either patient group**
Whole striatum	0.01521±0.00121	0.01465±0.00112	0.01351±0.00135	0.9191
Associative striatum	0.01483±0.00131	0.01420±0.00126	0.01318±0.00136	0.7781
Limbic striatum	0.01439±0.00115	0.01411±0.00076	0.01315±0.00107	1.0344
Sensorimotor striatum	0.01647±0.00135	0.01596±0.00132	0.01446±0.00161	1.0189

Table 3 Subject characteristics of the studies of presynaptic dopamine capacity using radiolabeled DOPA

Authors	Controls		Patients					
	N (Male/Female)	Age (±s.d.)	N (Male/Female)	Age (±s.d.)	Antipsychotic treatment*	CPZ equivalents (mg/day) (±s.d.)	Total symptom score (±s.d.)	Presynaptic dopamine capacity in the striatum
Reith *et al* (1994)	13 (9/4)	36 (±13)	5 (5/0)	38 (±4)	4 naïve, 1 free for >3 years	-	PANSS: 58 (na)	Increased in patients
Hietala *et al* (1995)	8 (6/2)	27 (±7)	7 (4/3)	26 (±7)	all drug naïve	-	PANSS: 81 (±14)	Increased in patients
Dao-Castellana *et al* (1997)	7 (na)	25 (±5)	6 (na)	26 (±9)	2 naïve, 4 free for ≥4 months	-	PANSS: 94 (na)	Increased in patients
Hietala *et al* (1999)	13 (8/5)	30.4 (±9.4)	10 (4/6)	29.6 (±8.8)	All naïve	-	PANSS: 77.6 (na)	Increased in patients
Lindstrom *et al* (1999)	10 (8/2)	na	12 (10/2)	31 (na)	10 naïve, 2 drug free for >2 years	-	na	Increased in patients
Elkashef *et al* (2000)	13 (8/5)	34.6 (±10.8)	19 (15/4)	36.3 (na)	10 taking drugs (6 clozapine), 9 drug free	na	na	No difference between patients on medication and controls
Meyer-Lindenberg *et al* (2002)	6 (5/1)	34 (na)	6 (5/1)	35 (na)	all free for ≥6 weeks	-	na	Increased in patients
McGowan *et al* (2004)	12 (12/0)	38.3 (±7.1)	16 (16/0)	39.9 (±11.3)	All on antipsychotic treatment	663 (na)	CASH: 10.6 (na)	Increased in patients
Kumakura *et al* (2007)	15 (15/0)	37.3 (±6.4)	8 (8/0)	37.3 (±6.3)	3 naive, 6 free for ≥6 months	-	PANSS: 80.2 (±4.7)	Increased in patients
Nozaki *et al* (2009)	20 (10/10)	35.1 (±9.5)	18 (10/8)	35.6 (±7.4)	14 naïve, 4 free	-	PANSS: 79.2 (±21.4)	Increased in patients
Howes *et al* (2009b)	12 (8/4)	24.3 (±4.6)	7 (5/2)	36.0 (±14.7)	2 naive, 5 free for >8 weeks	-	PANSS: 61.7 (±31.0)	Increased in patients
Shotbolt *et al* (2011)	20(10/10)	39 (±14)	6 (na)	43 (±12)	All on antipsychotic treatment	216 (na)	PANSS: 56.8 (±25.4)	No difference between patients on medication and controls

Demjaha *et al* (2012)	12 (5/7)	44.2 (±8.9)	Treatment-Resistant: 12 (5/7) Treatment Responder: 12 (6/6)	Treatment-Resistant: 45.7 (±9.8) Treatment Responder: 44.0 (±11.9)	All on antipsychotic treatment	Treatment-Resistant: 396.1 (±157.5) Treatment Responder: 283.9 (±159.14)	PANSS: Treatment-Resistant: 104.3 (±10.6) Treatment Responder: 50.7 (±5.8)	No difference between Treatment-Resistant and controls Increased in Treatment Responder
Howes *et al* (2013)**	29(22/7)	29.3 (±7.5)	29 (26/3)	33.7 (±10.6)	5 naïve, 8 free for >3 months 16 on antipsychotic treatment	-	CASH: 77.6 (±47.6)	Increased patients

*: Patients treated with clozapine were included only in Elkashef et al. 2006

**:Fourteen antipsychotic treated patients and twelve controls in Howes et al 2013 have been included in McGowan et al 2004

Figure 1.

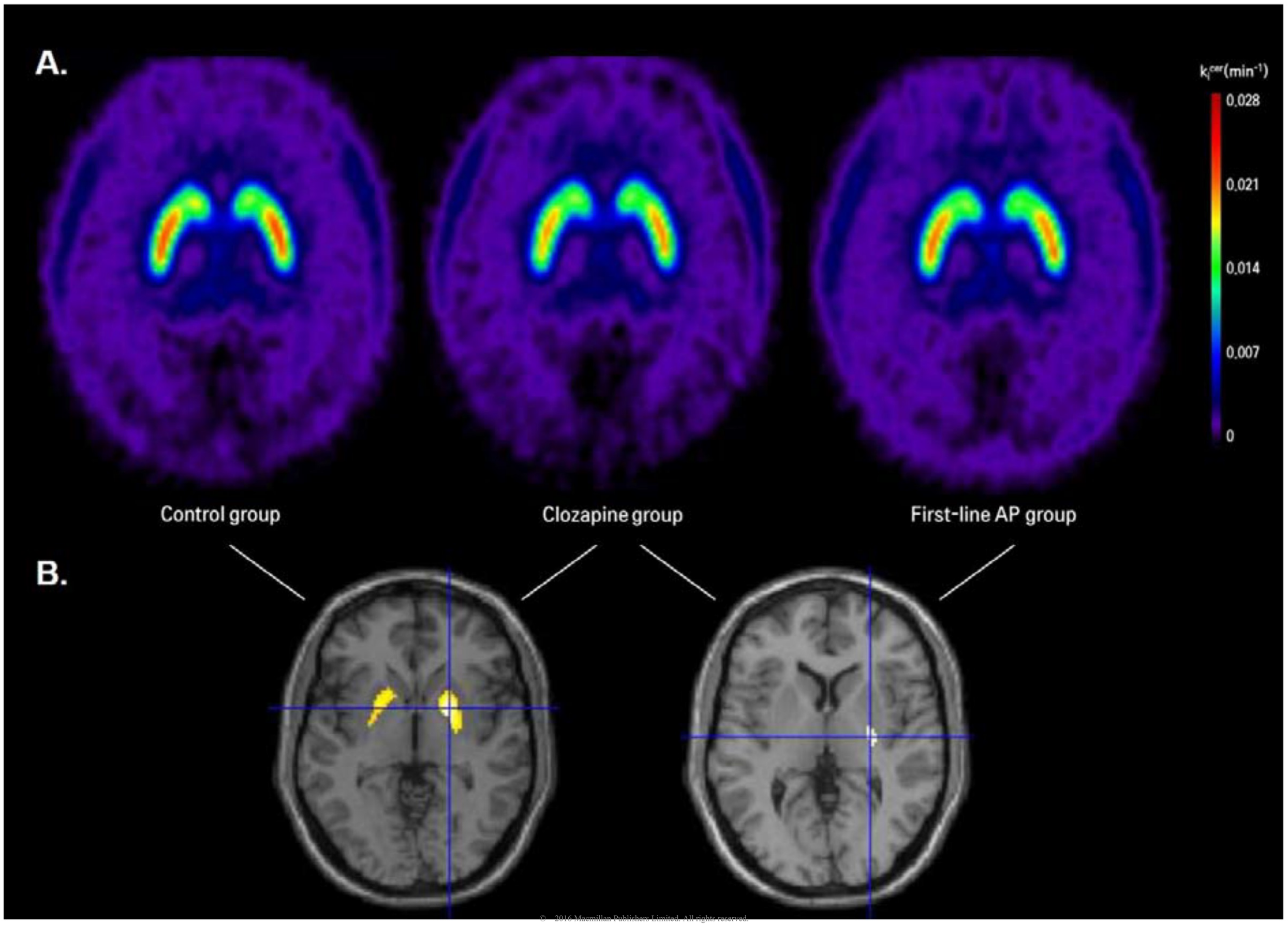

Figure 2.